Photochemical Conjugation and One-Pot Radiolabelling of Antibodies for Immuno-PET

Patra, Malay ; Eichenberger, Larissa S ; Fischer, Gregor ; Holland, Jason P

Abstract: Monoclonal antibodies (mAbs), immunoglobulin fragments, and other proteins are important scaffolds in the development of radiopharmaceuticals for diagnostic immuno-positron emission tomography (immuno-PET) and targeted radioimmunotherapy (RIT). Conventional methods for radiolabelling proteins with metal ions such as 68Ga, 64Cu, 89Zr, and 90Y require multi-step procedures involving pre-purification, functionalisation with a chelate, and subsequent radiolabelling. Standard coupling chemistries are time-consuming, difficult to automate, and involve synthesis, isolation, and storage of an intermediate, new molecular entity (the conjugated mAb) whose biochemical properties can differ from those of the parent protein. To circumvent these issues, we developed a photoradiochemical approach that uses fast, chemoselective, light-induced protein modification under mild conditions with novel metal-ion-binding chelates derivatised with aryl azide (ArN3) groups. Experiments show that one-pot photochemical conjugation and radiolabelling of formulated mAbs can be achieved in <20 min.

DOI: https://doi.org/10.1002/anie.201813287

A Journal of the Gesellschaft Deutscher Chemiker

Angewandte International Edition Chemie

GDCh

www.angewandte.org

Accepted Article

Title: Radiochemistry in a flash: photochemical conjugation and one-pot radiolabelling of antibodies for immuno-PET

Authors: Malay Patra, Larissa Eichenberger, Gregor Fischer, and Jason P. Holland

To be cited as: *Angew. Chem. Int. Ed.* 10.1002/anie.201813287
Angew. Chem. 10.1002/ange.201813287

Link to VoR: http://dx.doi.org/10.1002/anie.201813287
http://dx.doi.org/10.1002/ange.201813287

WILEY-VCH

10.1002/anie.201813287

WILEY-VCH

COMMUNICATION

Radiochemistry in a flash: photochemical conjugation and one-pot radiolabelling of antibodies for immuno-PET

Malay Patra,[a]† Larissa S. Eichenberger,[a] Gregor Fischer[b] and Jason P. Holland*[a]

Abstract: Monoclonal antibodies (mAbs), immunoglobulin fragments and other proteins are important scaffolds in the development of radiopharmaceuticals for diagnostic immuno-positron emission tomography (immuno-PET) and targeted radioimmunotherapy (RIT). Conventional methods for radiolabelling proteins with metal ions like ^{68}Ga, ^{64}Cu, ^{89}Zr, and ^{90}Y etc require multi-step procedures involving pre-purification, functionalisation with a chelate, and subsequent radiolabelling. Standard coupling chemistries are time consuming, difficult to automate, and involve synthesis, isolation and storage of an intermediate, new molecular entity (the conjugated mAb) whose biochemical properties can differ from those of the parent protein. To circumvent these issues, we developed a *photoradiochemical* approach that uses fast, chemoselective, light-induced protein modification under mild conditions with novel metal ion binding chelates derivatised with arylazide (ArN_3) groups. Experiments show that one-pot photochemical conjugation and radiolabelling of formulated mAbs can be achieved in <20 min.

Classical methods for radiolabelling proteins usually involve multiple steps in which the protein is first purified, coupled to a metal binding chelate, isolated, stored, and then radiolabelled.[1] Many coupling chemistries exist, but in the clinic, most radiolabelled mAb production relies on modification of either cysteine (thiolate) resides using maleimido-based reagents, or functionalisation of the lysine ε-NH_2 side-chain by using *N*-hydroxysuccinimide (NHS) or isothiocyanate (NCS) reagents.[2] Modern routes that employ enzymatic coupling[3–5] or strain-promoted alkene-alkyne 'click'-based[6–8] chemistry are advancing, but from a translational perspective, they present additional challenges for use in Good Manufacturing Processes.

An ideal radiolabelling method should be simple, fast, and involve a single-step starting from well-characterised, stable precursors, with no isolated intermediates. As clinical radiopharmaceutical productions use high levels of radioactivity, full automation is desirable. Time constraints imposed by radioactive decay also mean that chemical kinetics are crucial in deciding if a new reaction is suitable for use in radiochemistry.[9] Taking these points into account, we postulated that photochemical methods could be an attractive alternative to thermochemical processes that are commonly used to radiolabel immunoglobulins, other proteins, peptides and small-molecules.

Photoaffinity labelling (PAL) of proteins is well-established and a rich array of photoactivatable groups have been reported.[10–12] PAL reagents are often built around benzophenones, diazirines and substituted arylazides (ArN_3). Irradiation with light (λ ~200 to ~420 nm), generates radical, carbene or nitrene intermediates that have observed lifetimes in the picosecond (ps) to microsecond (μs) range. Such extreme reactivity means that mechanistically, PAL usually requires pre-association of the photoactive ligand with the target protein to avoid non-productive background reactions. Pre-formation of the non-covalent ligand-protein complex facilitates first-order intramolecular bond formation but restricts the standard PAL concept to systems that self-assemble. If reagents and conditions can be found whereby bimolecular coupling of a photo-induced intermediate to proteins occurs faster than background reactions, then photochemical conjugation could be generalised for a more wide-spread use in radiochemistry.

Surprisingly few examples exist where experiments combined photochemistry with radiochemistry. In pioneering work, Sykes *et al.* used photoactivation of an antibody to generate reactive sulfhydryl groups which facilitated ^{99m}Tc and ^{188}Re radiolabelling.[13,14] The same approach was also used by Stalteri and Mather.[15] Hashizume *et al.* prepared [^{18}F]pentafluorophenyl azide *via* isotopic exchange.[16] Wester *et al.* reported that ultra-violet irradiation of 4-azidophenacyl-[^{18}F]fluoride ([^{18}F]APF) gave radiolabelled proteins in ~15% to ~35% radiochemical yield (RCY).[17] Lange *et al.* used 3-azido-5-nitrobenzyl-[^{18}F]fluoride to radiolabel an oligonucleotide aptamer.[18] Pandurangi *et al.* extended the concept to complexes of ^{109}Pd, Re, and ^{99m}Tc.[19–22] Finally, Nishikawa *et al.* radiolabelled plasmid DNA with ^{111}In using photoactivatable 4-(*p*-azidosalicylamido)butylamine diethylenetriaminepentaacetic acid (DTPA-ASBA).[23] Since these initial studies, photochemical methods have remained relatively untouched by radiochemists.

Here, we show that photochemical conjugation can be used to radiolabel mAbs with ^{68}Ga ($t_{1/2}$ = 67.71 min.) *via* an ArN_3-functionalised chelate (NODAGA-PEG_3-ArN_3, compound **5**, Scheme 1). The method works *via* a conventional two-step, pre-conjugation and radiolabelling pathway, and also by a one-pot, pre-radiolabelling route. Remarkably, photochemical conjugation was successful using formulated antibody solutions (Herceptin™),

[a] Dr M. Patra, L. Eichenberger, Prof. Dr J. P. Holland*
University of Zurich, Department of Chemistry, Winterthurerstrasse 190, Zurich, CH-8057, Switzerland
Correspondence to:
E-mail: jason.holland@chem.uzh.ch
Tel: +41.44.63.53.990
Website: www.hollandlab.org
[b] Dr Gregor Fischer
University of Zurich, Laboratory Animal Services Center
† Present address: Department of Chemical Sciences, Tata Institute of Fundamental Research, Homi Bhabha Road, Mumbai 400005, India

Supporting information for this article is given *via* a link at the end of the document.

10.1002/anie.201813287

without pre-purification of the mAb. The speed and simplicity of this photoradiochemical route renders the method suitable for automation.

Insert Scheme 1

Scheme 1. Photochemical conjugation and radiolabelling of antibodies. (Top) Conventional two-step pathway involving pre-conjugation and radiolabelling. (Bottom) One-pot pathway involving pre-radiolabelling then photochemical conjugation. (Centre) Simultaneous, one-pot photoradiochemical labelling.

The photoactivatable ligand, NODAGA-PEG$_3$-ArN$_3$ (**5**) was synthesised in three steps *via* standard chemical transformations (overall yield 19.4%; Supporting Information Scheme S1, Figures S2 – S16). *Para*-substituted ArN$_3$ was selected as the photoreactive group.[10,24] Irradiation of ArN$_3$ releases N$_2$(g) forming a singlet arylnitrene (^{1}A$_2$ ground state), which at room temperature undergoes extremely fast (k_1 ~10^9 s^{-1}; lifetime, τ ~1 ns) intramolecular rearrangement to give benzazirine or ketenimines intermediates.[25] Unlike the radical or carbene species produced from irradiation of benzophenones and diazirines, respectively, ketenimines react relatively slowly with oxygen, protons and water, but undergo rapid nucleophilic addition with primary or secondary amines.[26,27] Thus, substituted arylazides are a suitable starting point for developing bimolecular photoradiochemistry.

Compound **5** readily formed coordination complexes with ^{nat}Ga^{3+} and radioactive ^{68}Ga^{3+} ions (Figures S17 – S20). Both the free ligand (**5**) and metal complexes ($^{nat/68}$Ga-**5**) were found to react upon irradiation with three different light sources (λ_{max} ~365 nm; Figures S21 – S23). The rate of photodegradation of compounds **5** and $^{nat/68}$Ga-**5** was proportional to light intensity and obeyed the anticipated first-order kinetics with, in each case, one major product observed by high-performance liquid chromatogram (HPLC). High-resolution mass spectrometry confirmed that irradiation of compound **5** led to the formation of NODAGA-PEG$_3$-azepin-2-ol which was likely produced *via* reaction between the photo-induced intermediates and water.

After confirming that compound **5** and $^{nat/68}$Ga-**5** were photoactive, trastuzumab (isolated by size-exclusion chromatography [SEC] from Herceptin^{TM}) was pre-incubated with the ligand, irradiated for 30 min. at room temperature, re-purified, and then radiolabelled with [^{68}Ga][Ga(H$_2$O)$_6$]Cl$_3$(aq.) under standard conditions. After reaction, the crude mixture was purified by preparative PD-10-SEC methods. Aliquots of the crude and purified samples were analysed by radioactive-instant thin-layer chromatography (radio-iTLC), analytical PD-10-SEC, and by SEC-ultra high-performance liquid chromatography (SEC-UHPLC; Figure 1, Table S1). Even though the photochemical step was performed under non-optimised conditions, radiolabelling of NODAGA-azepin-trastuzumab was complete in <15 min. giving [^{68}Ga]GaNODAGA-azepin-trastuzumab in a radiochemical conversion >98% (RCC; n = 6), a molar activity A_m of 16.3 ± 4.1 MBq/nmol (n = 8), and a final radiochemical purity of >98% by SEC-UHPLC. Starting from a 50-fold molar excess of chelate with respect to protein in the photochemical step, the measured accessible chelate-to-mAb ratio in the radiolabelled product was 0.39 ± 0.10 (n = 8). Control reactions confirmed that no non-specific binding occurred between ^{68}Ga^{3+} ions and the mAb. Trastuzumab was also stable under the photochemical conjugation and radiolabelling conditions (Figures S24 – S26).

Insert Figure 1

Figure 1. Characterisation data for the radiochemical synthesis of [^{68}Ga]GaNODAGA-azepin-trastuzumab. (A) Radio-iTLC chromatograms, (B) analytical PD-10-SEC elution profiles, and (C) SEC-UHPLC chromatograms of the crude and purified product.

Next, we optimised conditions for the photochemical conjugation between compound **5** and trastuzumab. As the conjugation mechanism relies on the nucleophilicity of an amine, the reaction pH was adjusted between 4.5 – 9.5 using NaHCO$_3$. Radiolabelling of crude photochemically coupled samples and subsequent chromatographic analyses showed that the RCC increased from ~1.0% at pH4.5 to ~17.8% at pH8.5 (Table S2, Figure S27). Further tests showed that with increasing light intensity, the photochemical conjugation step could be reduced <5 min. without compromising reaction efficiency. Also, with efficient mixing, the radiolabelling step was complete in <5 min. The final chelate-to-antibody ratio was reassessed after photochemical conjugation at pH8.5 and a linear relationship was found between the measured number of accessible chelates bound to the mAb and the initial chelate-to-mAb ratio (Table S3, Figure S28). Assuming that all accessible chelates were radiolabelled, and using the RCC and the measured fraction of photoreacted starting material, the photochemical conjugation efficiency for trastuzumab functionalisation with compound **5** was estimated to be ϕ_r = 0.351 (equivalent to a reaction quantum yield). Most efficient thermochemical coupling methods have yields in the range 30% – 80%[28–30] which makes the photochemical route competitive with existing technology.

The reaction was then scaled up and the product was subjected to standard chemical and biochemical tests *in vitro*. Using an initial chelate-to-mAb ratio of 5:1, [^{68}Ga]GaNODAGA-azepin-trastuzumab in sterile PBS was prepared in a decay corrected RCY of 80.1 ± 4.1%, with RCP >98%, and a molar activity, A_m of 38.4 ± 0.7 MBq/nmol (Figure S29). Stability tests showed no change in RCP after incubating the product in excess EDTA or in human serum at 37 °C for >2 h (Figures S30 – S31, Table S4). Saturation binding (Lindmo) assays using SK-OV-3 cells (HER2/*neu* positive) also confirmed that [^{68}Ga]GaNODAGA-azepin-trastuzumab was biochemically viable with an immunoreactive fraction of 70.7 ± 4.9%, which is comparable to previous reports on radiolabelled trastuzumab (Figure S32).[30]

PET imaging and biodistribution experiments were then performed in athymic nude mice (n = 4 animals/group) bearing subcutaneous SK-OV-3 tumours to measure the pharmacokinetic (PK) profile and binding specificity of [^{68}Ga]GaNODAGA-azepin-trastuzumab *in vivo* (Figure 2, Figures S33 – S38, Table S5). Measurements of total radioactivity in the animals found that the effective half-life ($t_{1/2}$(eff) of [^{68}Ga]GaNODAGA-azepin-

10.1002/anie.201813287

trastuzumab was 68.0 ± 1.8 min., equivalent to the physical half-life of the radionuclide and consistent with the expected PK profile of radiolabelled mAbs (Figure S39).[30] Temporal PET imaging revealed that [^{68}Ga]GaNODAGA-azepin-trastuzumab remained in circulation for >6 h post-injection with most activity visible in the blood pool component of the heart. Tumours of the normal group of animals were readily visualised by PET. Competitive binding studies using co-injection of a blocking dose of non-radiolabelled trastuzumab (low molar activity formulation, A_m of 1.85 ± 0.01 MBq/nmol of protein) confirmed that tumour uptake was specific and that the product remained biologically active *in vivo*. Biodistribution analysis *ex vivo* corroborated the PET data and found radioactivity accumulation of 16.5 ± 1.0 %ID/g in the normal tumours compared with 8.9 ± 1.1 %ID/g in tumours of the blocking group (Student's *t*-test *P*-value < 0.001).

Insert Figure 2

Figure 2. [^{68}Ga]GaNODAGA-azepin-trastuzumab PET images recorded in mice bearing SK-OV-3 tumours on the right flank. T = tumour, H = heart, L = liver, Sp = spleen.

Encouraged by the results, we tested the photoradiochemical reaction further by using a one-pot approach. Compound **5** was pre-radiolabelled with ^{68}Ga, re-buffered to pH ~8.0, mixed with pre-purified trastuzumab and then irradiated (Figure 3). Analytical methods confirmed that the one-pot pre-radiolabelling approach could be completed in <20 min. total time (including radiolabelling, photochemical conjugation and purification) to give [^{68}Ga]GaNODAGA-azepin-trastuzumab in sterile PBS with a decay corrected RCY of 33.9 ± 0.7% (n = 3). Development of this fast and efficient one-pot route simplifies the production of radiolabelled proteins by removing the need to synthesise, isolate and store the pre-functionalised intermediate under GMP conditions. In addition, the one-pot route can be automated easily using modular radiosynthesis units.

Insert Figure 3

Figure 3. Characterisation data for the one-pot photoradiochemical synthesis of [^{68}Ga]GaNODAGA-azepin-trastuzumab from pre-purified mAb. (A) Radio-iTLC chromatograms, (B) analytical PD-10-SEC elution profiles, and (C) SEC-UHPLC chromatograms of the crude and purified product.

Finally, the one-pot photoradiochemistry approach was tested using fully formulated Herceptin™ (i.e. non-purified trastuzumab). Clinical preparations of antibodies are typically stabilised by the addition of salts, amino acids, anti-oxidants and surfactants. Herceptin™ contains histidine, α,α-trehalose dehydrate and polysorbate 20. Traditional coupling methods do not tolerate such additives which necessitates pre-purification of the mAb (usually from a GMP source). Removing the stabilisers risks damaging the protein, and isolation/storage of an intermediate species raises concerns regarding the long-term biological integrity of the radiolabelling precursor with respect to the parent compound. Methods that allow direct radiolabelling of the formulated GMP-grade mAbs could potentially redefine the way in which radiopharmaceuticals are prepared for immuno-PET and RIT. Experiments showed that the photoradiochemical approach using Herceptin™ produced [^{68}Ga]GaNODAGA-azepin-trastuzumab in a decay-corrected RCY of 23.3 ± 3.4% (n = 3, Figure S40). Interestingly, histidine had only a small impact on the RCY. Additional experiments also confirmed that the one-pot approach was applicable for radiolabelling other proteins including a scFv-Fc fragment and human serum albumin (Figure S41).

From a pharmacokinetic standpoint, the combination of ^{68}Ga with long-circulating, full-length mAbs is sub-optimal but this radionuclide confirms the proof-of-concept and is useful for radiolabelling lower molecular weight species like immunoglobulin fragments and peptides. Compound **5** can also form complexes with other radionuclides including ^{64}Cu. Based on data reported here, we have expanded the approach by synthesising a range of photoactivatable chelates for radiolabelling with ^{64}Cu, ^{89}Zr, ^{90}Y, ^{111}In, ^{177}Lu, ^{225}Ac and others. For systems where the pH window for radiolabelling is >7 - 8 (for example, [^{89}Zr][Zr(C_2O_4)$_4$]$^{4-}$(aq.) complexation by desferrioxamine B [DFO]), a simultaneous photoradiochemical process is feasible (Scheme 1). Experiments continue to investigate the effects of linker modification and ArN_3 substitution on bimolecular photochemical conjugation efficiency, as well as chemical scope of photoradiochemistry using immunoglobulin fragments, proteins, peptides, nanoparticles and small-molecules.

Experimental Section

Full experimental details and characterisation data are provided in the Supporting Information.

Acknowledgements

JPH thanks the Swiss National Science Foundation (SNSF Professorship PP00P2_163683), the European Research Council (ERC-StG-2015, NanoSCAN – 676904), the Swiss Cancer League (Krebsliga Schweiz; KLS-4257-08-2017), and the University of Zurich (UZH) for financial support. We thank all members of the Radiochemistry and Imaging Science group at UZH, and especially Dr Eliane Fischer, Dr Benjamin Probst, Allan Murphy and Simon Klingler.

Keywords: Radiochemistry • photochemistry • positron emission tomography • antibody conjugates • arylazides

[1] E. Boros, J. P. Holland, *J. Label. Compd. Radiopharm.* **2018**, *61*, 652–671.

[2] P. Dennler, E. Fischer, R. Schibli, *Antibodies* **2015**, *4*, 197–224.

[3] S. Jeger, K. Zimmermann, A. Blanc, J. Grünberg, M. Honer, P. Hunziker, H. Struthers, R. Schibli, *Angew. Chemie - Int. Ed.* **2010**, *49*, 9995–9997.

[4] P. Dennler, A. Chiotellis, E. Fischer, D. Brégeon, C. Belmant, L. Gauthier, F. Lhospice, F. Romagne, R. Schibli, *Bioconjug. Chem.*

2014, *25*, 569–578.

[5] B. M. Zeglis, C. B. Davis, R. Aggeler, H. C. Kang, A. Chen, B. J. Agnew, J. S. Lewis, *Bioconjug. Chem.* **2013**, *24*, 1057–1067.

[6] R. Rossin, P. R. Verkerk, S. M. Van Den Bosch, R. C. M. Vulders, I. Verel, J. Lub, M. S. Robillard, *Angew. Chemie - Int. Ed.* **2010**, *49*, 3375–3378.

[7] N. K. Devaraj, R. Upadhyay, J. B. Haun, S. A. Hilderbrand, R. Weissleder, *Angew. Chemie - Int. Ed.* **2009**, *48*, 7013–7016.

[8] N. K. Devaraj, R. Weissleder, *Acc. Chem. Res.* **2011**, *44*, 816–827.

[9] J. P. Holland, *Chem. - a Eur. J.* **2018**, *In press*, DOI 10.1002/chem.201803261.

[10] P. Klán, J. Wirz, *Photochemistry of Organic Compounds: From Concepts to Practice*, **2009**.

[11] V. Chowdhry, F. H. Westheimer, *Annu. Rev. Biochem.* **1979**, *48*, 293–325.

[12] H. Bayley, J. R. Knowles, *Methods Enzymol.* **1977**, *46*, 69–114.

[13] T. R. Sykes, T. K. Woo, R. P. Baum, P. Qi, A. Noujaim, *J. Nucl. Med.* **1995**, *36*, 1913–1922.

[14] T. R. Sykes, V. A. Somayaji, S. Bier, T. K. Woo, C. S. Kwok, V. Snieckus, A. Noujaim, *Appl. Radiat. Isot.* **1997**, *48*, 899–906.

[15] M. A. Stalteri, S. J. Mather, *Eur. J. Nucl. Med.* **1996**, *23*, 178–187.

[16] K. Hashizume, N. Hashimoto, Y. Miyake, *J. Org. Chem.* **1995**, *60*, 6680–6681.

[17] H. J. Wester, K. Hamacher, G. Stöcklin, *Nucl. Med. Biol.* **1996**, *23*, 365–372.

[18] C. W. Lange, H. F. VanBrocklin, S. E. Taylor, *J. Label. Compd. Radiopharm.* **2002**, *45*, 257–268.

[19] R. S. Pandurangi, S. R. Karra, K. V. Katti, R. R. Kuntz, W. a. Volkert, *J. Org. Chem.* **1997**, *62*, 2798–2807.

[20] R. S. Pandurangi, P. Lusiak, R. R. Kuntz, W. A. Volkert, J. Rogowski, M. S. Platz, *J. Org. Chem.* **1998**, *63*, 9019–9030.

[21] R. Rajagopalan, R. R. Kuntz, U. Sharma, W. A. Volkert, R. S. Pandurangi, *J. Org. Chem.* **2002**, *67*, 6748–6757.

[22] R. S. Pandurangi, S. R. Karra, R. R. Kuntzl, W. A. Volkert, *Photochem. Photobiol.* **1997**, *65*, 208–221.

[23] M. Nishikawa, T. Nakano, T. Okabe, N. Hamaguchi, Y. Yamasaki, Y. Takakura, F. Yamashita, M. Hashida, *Bioconjug. Chem.* **2003**, *14*, 955–961.

[24] N. P. Gritsan, M. S. Platz, *Chem. Rev.* **2006**, *106*, 3844–3867.

[25] N. P. Gritsan, Z. Zhu, C. M. Hadad, M. S. Platz, *J. Am. Chem. Soc.* **1999**, *121*, 1202–1207.

[26] M. S. Platz, *Acc. Chem. Res.* **1995**, *28*, 487–492.

[27] G. W. Preston, A. J. Wilson, *Chem. Soc. Rev.* **2013**, *42*, 3289–3301.

[28] M. J. W. D. Vosjan, L. R. Perk, G. W. M. Visser, M. Budde, P. Jurek, G. E. Kiefer, G. A. M. S. van Dongen, *Nat. Protoc.* **2010**, *5*, 739–743.

[29] J. P. Holland, V. Divilov, N. H. Bander, P. M. Smith-Jones, S. M. Larson, J. S. Lewis, *J. Nucl. Med.* **2010**, *51*, 1293–1300.

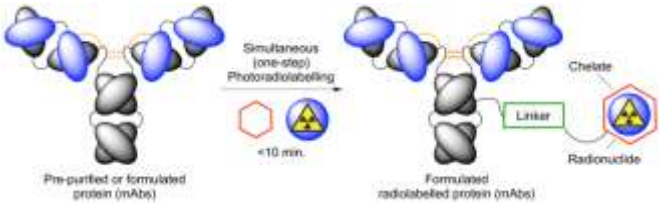
[30] J. P. Holland, E. Caldas-Lopes, V. Divilov, V. a Longo, T. Taldone, D. Zatorska, G. Chiosis, J. S. Lewis, *PLoS One* **2010**, *5*, e8859.

10.1002/anie.201813287

WILEY-VCH

Entry for the Table of Contents

COMMUNICATION



Radiochemistry in a flash! A photochemical conjugation and radiolabelling approach is described that allows rapid, one-pot synthesis of radiolabelled monoclonal antibodies (and other proteins/peptides) for use in immuno-positron emission tomography (immuno-PET) and targeted radioimmunotherapy.

*Malay Patra, Larissa S. Eichenberger, Gregor Fischer and Jason P. Holland**

Page No. – Page No.

Radiochemistry in a flash: photochemical conjugation and one-pot radiolabelling of antibodies for immuno-PET

10.1002/anie.201813287

WILEY-VCH

COMMUNICATION

Scheme 1.

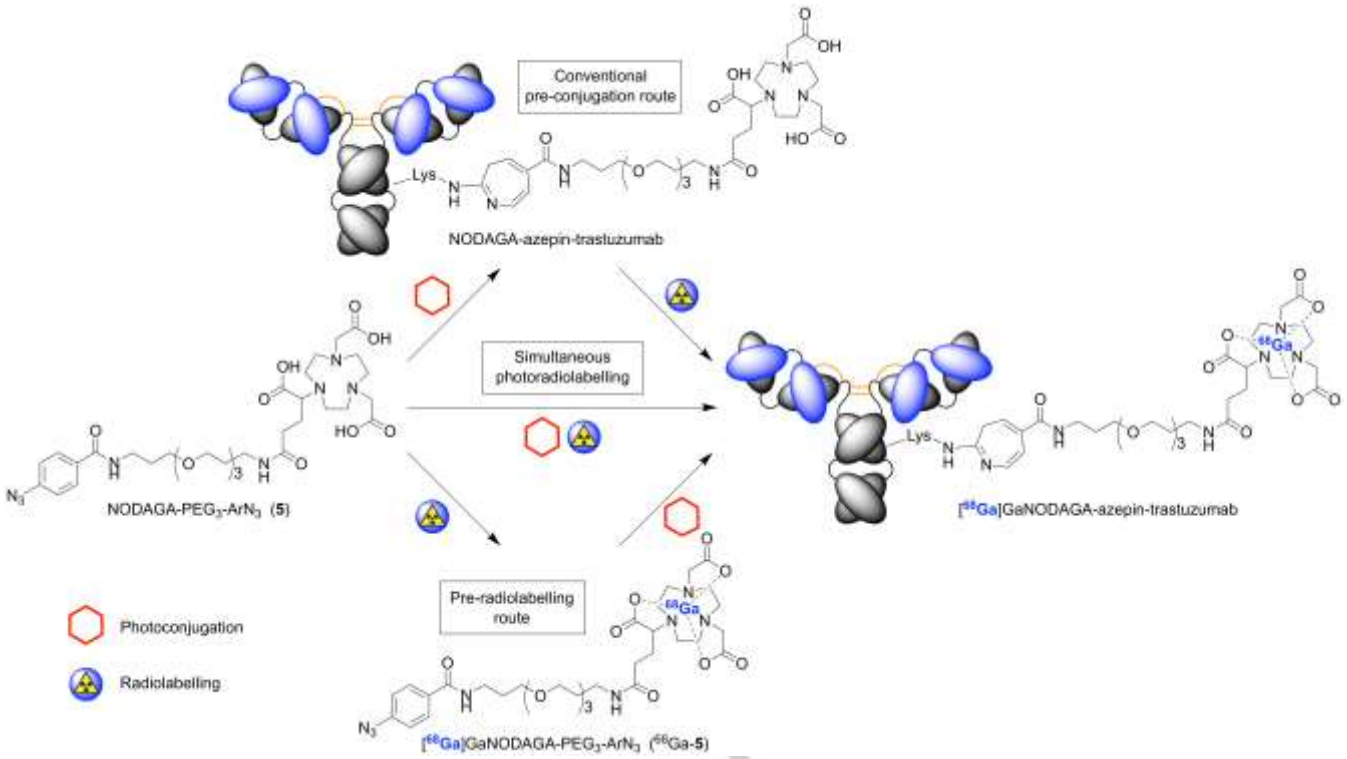
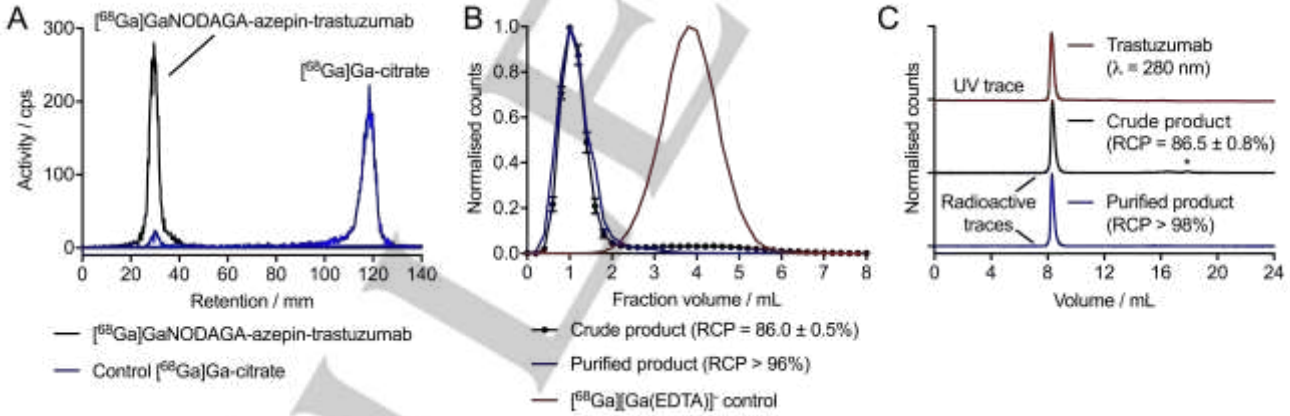


Figure 1.



10.1002/anie.201813287

WILEY-VCH

COMMUNICATION

Figure 2.

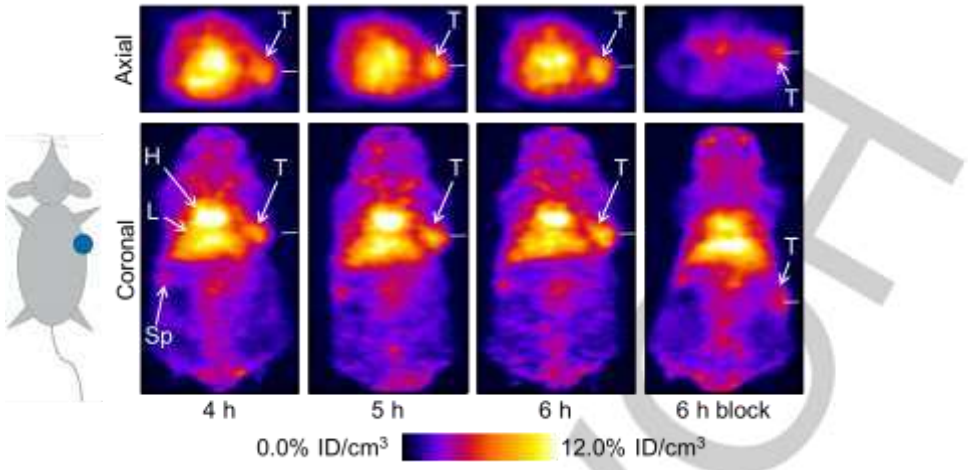
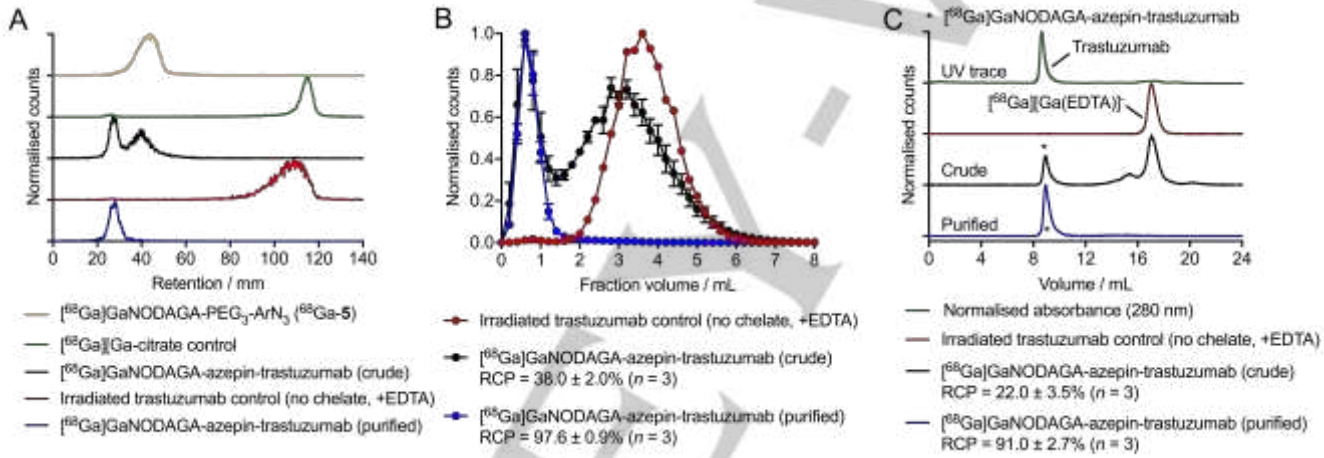


Figure 3.



10.1002/anie.201813287

WILEY-VCH

COMMUNICATION

Frontispiece/Cover art suggestion